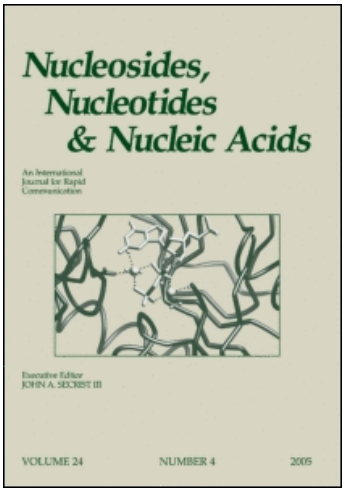

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The Effect of INA [(*R*)-1-*O*-(1-Pyrenylmethyl)Glycerol] Insertions on the Structure and Biological Activity of a G-Quadruplex from a Critical *Kras* G-Rich Sequence

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THE EFFECT OF INA [(*R*)-1-*O*-(1-PYRENYLMETHYL)GLYCEROL] INSERTIONS ON THE STRUCTURE AND BIOLOGICAL ACTIVITY OF A G-QUADRUPLEX FROM A CRITICAL *KRAS* G-RICH SEQUENCE

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□ *Quadruplex-forming oligonucleotides containing INA [(R)-1-O-(1-pyrenylmethyl)glycerol] insertions have been designed and studied for their capacity to inhibit the expression of the KRAS oncogene in pancreatic adenocarcinoma cells. It is found that INA can influence the folding topology of the G-quadruplex. The oligonucleotides forming the most stable G-quadruplex (ODN-637) is found to exhibit the highest bioactivity.*

Keywords G-quadruplex DNA; INA-conjugated oligonucleotides; *KRAS* promoter

INTRODUCTION

A nuclease hypersensitive element (NHE) located upstream from the major transcription initiation site in the human *KRAS* oncogene is able to assume a G-quadruplex conformation.[1] This unusual DNA structure is formed in a promoter region that is recognized by nuclear proteins.[1] By affinity chromatography and SDS-PAGE we found that three proteins (110 kDa, 60 kDa and 32 kDa) recognize NHE in the double-stranded as well as intramolecular folded quadruplex forms. Recent data obtained in our laboratory strongly suggest that the *KRAS* G-quadruplex is probably a transcription repressor element (1 and unpublished data). Within this framework we hypothesised a new molecular approach to inhibit the expression of the *KRAS* oncogene. We reasoned that synthetic oligonucleotides mimicking quadruplex NHE should act as aptamers and deprive the *KRAS* promoter of proteins necessary for transcription. As proof-of concept we designed the following quadruplex-forming oligonucleotides:

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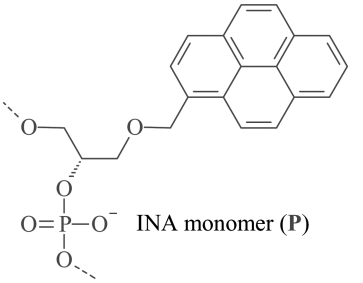
3′-GACGGAGGGGGAGAAGGGAGAAGGGTGTGGCG ODN-wt
3′-GACGGAGGGGGAGAAGGGAGAAGGG**P**TGTGGCG ODN-634
3′-GACGGAGGGGGAGAAGGGAGAA**P**GGGTGTGGCG ODN-637
3′-GACGG**P**AGGGGGAGAAGGGAGAAGGGTGTGGCG ODN-635
3′-GACGGAGG**P**GGGAGAAGGGAGAAGGGTGTGGCG ODN-638
P = [(*R*)-1-*O*-(1-Pyrenylmethyl)glycerol] (INA)



To enhance the thermal stability of the G-quadruplexes, we introduced one INA unit at different positions of ODN-wt [2]. According to DMS-footprinting and circular dichroism data, we proposed for the unmodified oligonucleotide ODN-wt a mixed parallel/antiparallel structure, composed by two G-tetrads and three loops (Figure 1). The CD spectrum of ODN-wt is characterized by two positive (260 and 295 nm) and one negative (240 nm) ellipticities and a thermal stability of 50°C, in 100 mM KCl (Figure 2, left).[3] The INA-conjugates ODN-634, ODN-635, ODN-638 and ODN-637 show CD spectra similar to that of ODN-wt and T_ms of 59, 56, 59, and 61°C, respectively. TypicalCD spectra as a function of temperature for ODN-wt and

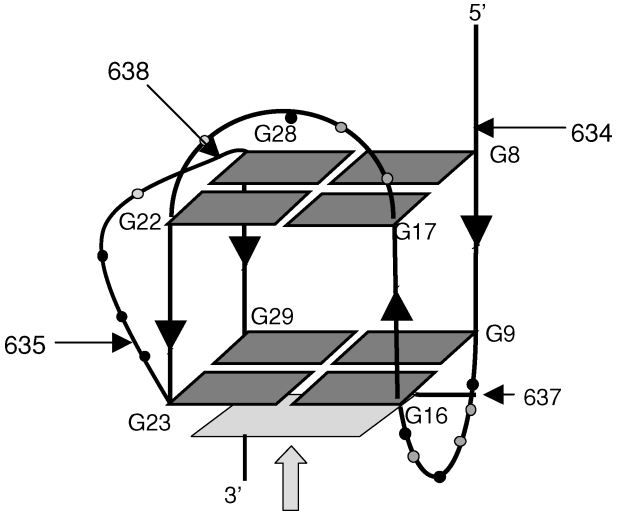


FIGURE 1 Putative structures of the G-quadruplex formed by a 32mer G-rich strand within NHE located in the *KRAS* promoter. The arrows indicate the position of INA in the conjugates.

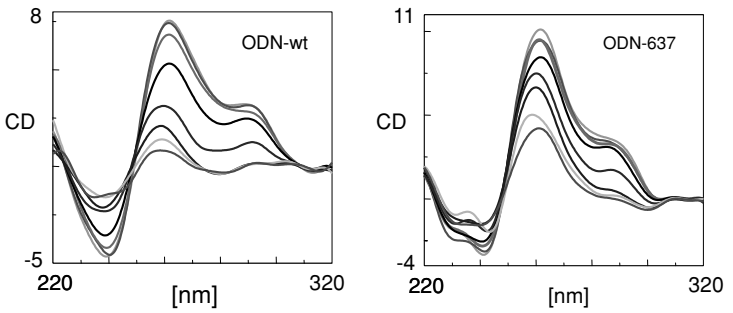


FIGURE 2 CD spectra of ODN-wt (left) and ODN-637 (right) in 50 mM Tris-HCl, pH 7.4, 100 mM KCl, obtained at increasing temperatures (25–90°C). Oligonucleotide concentration 5 μM, cell pathlength is 0.5 cm. Ellipticity is expressed in mdeg.

ODN-637 are shown in Figure 2, right. The enhanced stability of ODN-637 is due to the fact that when INA is inserted in a 6-nt loop, it probably stacks on the external G-tetrad more efficiently than when it is inserted in the other positions, including the 5′ flanking end (ODN-634). This is suggested by photocleavage experiments with TMPyP4.[1,5] The designed G-quadruplex forming oligonucleotides were tested for their capacity to interfere with *KRAS* transcription and inhibit cell growth. The INA-conjugates (1 μM), complexed with lipofectamine, were delivered to Panc-1 cells and after 24 and 48 hours mRNA was extracted. The mRNA extracts were analysed by real-time PCR and found that the INA conjugates, in particular ODN-637, promoted a significant reduction of the *KRAS* transcript level.

Moreover, we observed by MTT assays that ODN-637 induced a decline of cell growth, in a dose dependent manner. This antiproliferative effect was also observed, but in a weaker extent with, ODN-wt and ODN-634. The fact that the G-quadruplex oligonucleotides reduced *KRAS* transcripts and exhibited an antiproliferative activity correlates with the transcription model proposed for the *KRAS* gene.[1]

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